adenoviral vectors can be propagated in high titer and transfect non-replicating cells. Human 293 cells, which are human embryonic kidney cells transformed with adenovirus E1A/E1B genes, typify useful permissive cell lines. However, other cell lines which allow replication-deficient adenoviral vectors to propagate therein can be used, including HeLa cells.—

REMARKS

Attached hereto is a marked-up version of the changes made to the specification by the current amendment. The attached page is captioned "Version with markings to show changes made".

In the unlikely event that the transmittal letter is separated from this document and/or the Patent Office determines that an extension and/or other relief is required, Applicant petitions for any required relief including extensions of time and authorizes the Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 50-1189**, referencing attorney docket no. 126881209900. However, the Commissioner is not authorized to charge the cost of the issue fee to the Deposit Account.

Respectfully submitted,

Date: August 9, 2001

By: _____
Antoinette F. Konski
Reg. No. 34,202

McCutchen, Doyle, Brown & Enersen
Three Embarcadero Center, Suite 1800
San Francisco, CA 94111
Telephone: (650) 849-4950
Facsimile: (650) 849-4800

In the Specification:

Paragraph beginning at line 16 of page 2 has been amended as follows:

Conventional methods to generate TILs involve mincing tumor biopsy tissue and culturing the cell suspension *in vitro* in the presence of the T cell growth factor interleukin-2 (IL-2). Over a period of several days, the combination of the tumor cells and IL-2 can stimulate the proliferation of tumor specific T cells at the expense of tumor cells. In this way, the T cell population is expanded. The T cells derived from the first expansion are subsequently mixed with either mitomycin C-treated or irradiated tumor cells and cultured *in vitro* with IL2 to promote further proliferation and enrichment of tumor reactive T cells. After several rounds of *in vitro* expansion, a potent anti-tumor T cell population can be recovered and used to identify tumor antigens via conventional but tedious expression cloning methodology. ~~Kawakani~~ Kawakami Y. et al. (1994) Proc. Natl. Acad. Sci. USA 91(9):3515-3519.

Paragraph beginning at line 26 of page 16 has been amended as follows:

A "viral vector" is defined as a recombinantly produced virus or viral particle that comprises a polynucleotide to be delivered into a host cell, either *in vivo*, *ex vivo* or *in vitro*. Examples of viral vectors include retroviral vectors, adenovirus vectors, adeno-associated virus vectors, alphavirus vectors and the like. Alphavirus vectors, such as Semliki Forest virus-based vectors and Sindbis virus-based vectors, have also been developed for use in gene therapy and immunotherapy. See, Schlesinger and Dubensky (1999) Curr Opin Biotechnol. 10(5):434-439 and ~~Zaks~~ Ying et al. (1999) Nat. Med. 5(7):823-827. In aspects where gene transfer is mediated by a retroviral vector, a vector construct refers to the polynucleotide comprising the retroviral genome or part thereof, and a therapeutic gene. As used herein, "retroviral mediated gene transfer" or "retroviral transduction" carries the same meaning and refers to the process by which a gene or nucleic acid sequences are stably transferred into the host cell by virtue of the virus entering the cell and integrating its genome into the host cell genome. The virus can enter the host cell via its normal mechanism of infection or be modified such that it binds to a different host cell surface receptor or ligand to enter the cell. As used herein, retroviral vector refers to a viral particle capable of introducing exogenous nucleic acid into a cell through a viral or viral-like entry mechanism.

Paragraph beginning at line 9 of page 24 has been amended as follows:

In one embodiment, the altered ligands of the invention have comparable affinity for MHC binding as the native ligand. It has been demonstrated that peptide:MHC class I binding properties correlate with immunogenicity (Sette A. et al. (1994) *J. Immunol.* 153(12):5586-5592;

van der Burg S.H. et al. (1996) J. Immunol. **156**:3308-3314). In a preferred embodiment, altered ligands of the invention bind to a TCR with a higher affinity than of that the "natural" ligand. Comparative binding of the native and altered ligands of the invention to an MHC class I molecule can be measured by methods that are known in the art and include, but are not limited to, calculating the affinity based on an algorithm (see, for example, Parker et al. (1992) J. Immunol. **149**:3580-3587) and experimentally determining binding affinity (see, for example, Tan et al. (1997) J. Immunol. Meth. **209**(1):25-36). For example, the relative binding of a peptide to a class I molecule can be measured on the basis of binding of a radiolabeled standard peptide to detergent-solubilized MHC molecules, using various concentrations of test peptides (*e.g.*, ranging from 100 mM to 1nM). MHC class I heavy chain and β2-microglobulin are coincubated with a fixed concentration (*e.g.*, 5 nM) radiolabeled standard (control) peptide and various concentrations of a test peptide for a suitable period of time (*e.g.*, 2 hours to 72 hours) at room temperature in the presence of a mixture of protease inhibitors. A control tube contains standard peptide and MHC molecules, but no test peptide. The percent MHC-bound radioactivity is determined by gel filtration. The IC_{50} (concentration of test peptide which results in 50% inhibition of binding of control peptide) is calculated for each peptide. Additional methods for determining binding affinity to a TCR are known in the art and include, but are not limited to, those described in al-Ramadi et al. (1992) J. Immunol. **155**(2):662-673; and Zuegel et al. (1998) J. Immunol. **161**(4):1705-1709.

Paragraph beginning at line 21 of page 28 has been amended as follows:

The following non classical amino acids may be incorporated in the peptides of the invention in order to introduce particular conformational motifs: 1,2,3,4-tetrahydroisoquinoline-3-carboxylate (~~Kazmierski~~ Kazmierski et al. (1991) J. Am. Chem. Soc. **113**:2275-2283); (2S,3S)-methyl-phenylalanine, (2S,3R)- methyl-phenylalanine, (2R,3S)-methyl-phenylalanine and (2R,3R)-methyl-phenylalanine (Kazmierski and Hruby (1991) Tetrahedron Lett. **32**(41):5769-5772); 2-aminotetrahydronaphthalene-2- carboxylic acid (Landis (1989) Ph.D. Thesis, University of Arizona); hydroxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (Miyake et al. (~~1989~~) (1984) J. Takeda Res. Labs. **43**:53-76) histidine isoquinoline carboxylic acid (Zechel et al. (1991) Int. J. Pep. Protein Res. **38**(2):131-138); and HIC (histidine cyclic urea), (Dharanipragada et al. (1993) Int. J. Pep. Protein Res. **42**(1):68-77) and ((1992) Acta. Cryst., Crystal Struc. Comm. **48(IV)**:1239-1241).

Paragraph beginning at line 3 of page 29 has been amended as follows:

The following amino acid analogs and peptidomimetics may be incorporated into a peptide to induce or favor specific secondary structures: LL-Acp (LL-3-amino-2-propenidone-6-carboxylic acid), a β-turn inducing dipeptide analog (Kemp et al. (1985) J. Org. Chem. **50**:5834-5838); β-sheet inducing analogs (Kemp et al. (1988) Tetrahedron Lett. **29**:5081-5082); β-turn inducing analogs (Kemp et al. (1988) Tetrahedron Lett. **29**:5057-5060); α-helix inducing analogs (Kemp et al. (1988) Tetrahedron Lett. **29**:4935-4938); γ-turn inducing analogs (Kemp et al. (1989) J. Org. Chem. **54**:109:115); analogs provided by the following references: Nagai and Sato

(1985) Tetrahedron Lett. **26**:647-650; and DiMaio et al. (1989) J. Chem. Soc. Perkin Trans. p. 1687; a Gly-Ala turn analog (Kahn et al. (1989) Tetrahedron Lett. **30**:2317); amide bond isostere (~~Clones~~ Jones et al. (1988) Tetrahedron Lett. **29**(31):~~38S3-38S6~~3853-3856); tretrazol (Zabrocki et al. (1988) J. Am. Chem. Soc. **110**:~~587S~~ 5875-5880); DTC (Samanen et al. (1990) Int. J. Protein Pep. Res. **35**:501:509); and analogs taught in Olson et al. (1990) J. Am. Chem. Sci. **112**:323-333 and Garvey et al. (1990) J. Org. Chem. ~~56:436~~ 55(3):936-940. Conformationally restricted mimetics of beta turns and beta bulges, and peptides containing them, are described in U.S. Patent No. 5,440,013, issued August 8, 1995 to Kahn.

Paragraph beginning at line 31 of page 35 has been amended as follows:

When the vectors are used for gene therapy *in vivo* or *ex vivo*, a pharmaceutically acceptable vector is preferred, such as a replication-incompetent retroviral or adenoviral vector. Pharmaceutically acceptable vectors containing the nucleic acids of this invention can be further modified for transient or stable expression of the inserted polynucleotide. As used herein, the term "pharmaceutically acceptable vector" includes, but is not limited to, a vector or delivery vehicle having the ability to selectively target and introduce the nucleic acid into dividing cells. An example of such a vector is a "replication-incompetent" vector defined by its inability to produce viral proteins, precluding spread of the vector in the infected host cell. An example of a replication-incompetent retroviral vector is LNL6 (Miller A.D. et al. (1989) BioTechniques **7**:980-990). The methodology of using replication-incompetent retroviruses for retroviral-mediated gene transfer of gene markers is well established (Correll et al. (1989) Proc. Natl. Acad. Sci. USA **86**:8912; Bordignon (1989) Proc. Natl. Acad. Sci. USA **86**:~~8912-52~~ 6748-6852; Culver K. (1991) Proc. Natl. Acad. Sci. USA **88**:3155; and Rill D.R. ~~(1991)~~ (1992) Blood **79**(10):2694-2700).

Paragraph beginning at line 17 of page 47 has been amended as follows:

2.   **Cytokine-release assay.** Analysis of the types and quantities of cytokines secreted by T cells upon contacting ligand-pulsed targets can be a measure of functional activity. Cytokines can be measured by ELISA or ELISPOT assays to determine the rate and total amount of cytokine production. (Fujihashi K. et al. (1993) J. Immunol. Meth. **160**:181; ~~Tanquay~~ Tanguay S. and Killion J.J. (1994) Lymphokine Cytokine Res. **13**:259).

Paragraph beginning at line 9 of page 48 has been amended as follows:

6.   **Tetramer staining.** MHC tetramers can be loaded with individual ligands and tested for their relative abilities to bind to appropriate effector T cell populations. (Altman J.D. et al. (1996) Science ~~274:5284~~ 274(5284):94-96).

52055893.1/19442-7000

Paragraph beginning at line 10 of page 50 has been amended as follows:

The recombinant adenoviral vectors based on the human adenovirus 5 (McGrory, W.J.et. al. (1988) Virology **163**:614-617 (1988))) are missing essential early genes from the adenoviral genome (usually E1A/E1B), and are therefore unable to replicate unless grown in permissive cell lines that provide the missing gene products *in trans*. In place of the missing adenoviral genomic sequences, a transgene of interest can be cloned and expressed in cells infected with the replication deficient adenovirus. Although adenovirus-based gene transfer does not result in integration of the transgene into the host genome (less than 0.1% adenovirus-mediated transfections result in transgene incorporation into host DNA), and therefore is not stable, adenoviral vectors can be propagated in high titer and transfect non-replicating cells. Human 293 cells, which are human embryonic kidney cells transformed with adenovirus E1A/E1B genes, typify useful permissive cell lines. However, other cell lines which allow replication-deficient adenoviral vectors to propagate therein can be used, including HeLa cells.